The electrophysiology of feeding circuits

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Obesity is quickly becoming one of the most common and debilitating disorders of the developed world. More than 60% of American adults are now overweight or obese, predisposing them to a host of chronic diseases. To understand the etiology of obesity, and to discover new therapies for obesity, we must understand the components of energy balance. In simple terms, energy intake (feeding) must equal energy expenditure (physical activity, basal metabolism and adaptive thermogenesis) for body weight homeostasis. To maintain homeostasis, neurocircuitry must sense both immediate nutritional status and the amount of energy stored in adipose tissue, and must be able to provide appropriate output to balance energy intake and energy expenditure. The brain receives various signals that carry information about nutritional and metabolic status including neuropeptide PYY3–36, ghrelin, cholecystokinin, leptin, glucose and insulin. Circulating satiety signals access the brain either by ‘leakage’ across circumventricular organs or transport across the blood–brain barrier. Signals can also activate sensory vagal terminals that innervate the whole gastrointestinal tract.

By whatever means central neurons receive feedback regarding feeding status (and this is the subject of continued debate), it is clear that the effects of peripheral satiety signals are mediated by specific signal transduction systems in identifiable areas of the brain that are known to control food intake and body weight. In particular, the arcuate nucleus of hypothalamus (ARH) is a crucial integrative center for modulating food intake [1,2]. The ARH contains at least two key populations of neurons that have opposite actions on food intake. One population expresses anorexigenic (appetite-suppressing) peptides, cocaine- and amphetamine-regulated transcript (CART) and alpha-melanocyte-stimulating hormone (α-MSH; derived from the proopiomelanocortin (POMC) precursor). The other population expresses the orexigenic (appetite-stimulating) peptides neuropeptide Y (NPY) and agouti-related peptide (AgRP) [3]. Neurons in the ARH subsequently innervate various second order hypothalamic targets (Figure 1) that express melanocortin-4 (MC4) and NPY receptors [4].

Several excellent reviews have been written on central feeding circuits [5–7]. These have primarily focused on how peripheral feeding-related signals engage hypothalamic circuits and alter the levels of various hypothalamic neuropeptides (e.g. NPY and melanocortins) or monoamine neurotransmitters (e.g. serotonin, noradrenaline and possibly dopamine) in the central nervous system (CNS). Most research investigating the central control of appetite focuses on the hypothalamus. We review the neural circuits within the hypothalamus and their connections to relevant brainstem circuits.

Importance of electrophysiology in understanding central feeding circuits

Many studies have evaluated the functional activity of central feeding circuits by studying the mRNA or protein expression of c-Fos or other immediate early gene products; for example, an increase in c-Fos mRNA and/or protein in individual neurons has been used as a marker of neuronal activation [8]. Although such changes in the expression of immediate early gene products suggest that the functional output of neural circuitry has changed, they do not provide direct information about neuronal activity. For example, several peripheral signals, notably leptin, inhibit neuronal activity, a response that cannot be easily detected by analyzing c-Fos levels. Neuronal activity can also be inferred by analyzing changes in the concentration of intracellular Ca2+ detected by fluorescent dyes that alter their spectral resolution.

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A crucial component that is missing in both mRNA and Ca2+ imaging studies is the activity 'pattern' of individual neurons. Indeed, the activity pattern of individual neurons might be essential in determining neurotransmitter release. Bursts of action potentials, which more effectively stimulate neuropeptide release [9], cannot be distinguished from fast, repetitive firing in c-Fos studies. Likewise, changes in the regional levels of anorexigenic or orexigenic neuropeptide mRNA might not be related to changes in the synaptic release of these neuropeptides. Ultimately, to understand how feeding-related signals relay appropriate information to various brain regions, the direct effect of such signals on neuronal activity must be assessed.

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Most of the electrophysiological data that we review here are from recordings of individual neurons made after the application of feeding-related signals to brain slices. This type of preparation allows an assessment of the effect of various signals on individual neurons in systems in which at least part of the neural circuitry is intact. By combining data from slice preparations with in vivo studies, our goal is to build a working ‘circuit diagram’ of feeding pathways. For brevity, we have chosen to review the electrophysiological effects of only a few feeding-related signals on neurons in various nuclei in the brain. Inclusion of these factors does not imply their preeminence in feeding circuitry; similarly, the exclusion of other factors does not suggest that those factors are insignificant. Rather, we have selected the feeding-related signals discussed below because we know more about them and because a synthesis of their actions is overdue.

**Electrophysiology of leptin**

In addition to regulating long-term energy balance by its genomic effects on NPY/AgRP and POMC neurons, leptin has rapid electrophysiological effects on these two neuronal populations (Box 1). Because POMC and NPY/AgRP neurons in the ARH show dense expression of the leptin receptor [10,11] and are the source of potent feeding-related neuropeptides, our group [2,12] has carried out electrophysiological studies on brain slice preparations taken from transgenic mice that have been engineered to express fluorescent proteins in these specific cell types. Using these models, we have shown that leptin rapidly increases the frequency of action potentials in POMC neurons by two distinct mechanisms: first, leptin causes direct depolarization through a nonspecific cation channel; and second, leptin causes a decrease in inhibitory GABA-mediated tone onto POMC cells by local orexigenic neurons expressing NPY and GABA (Figure 2) [2]. The second mechanism – that is, activation of POMC neurons by ‘disinhibition’ of local GABAergic tone – recurs as a common mechanism of modulating neuronal activity that is used by several other feeding-related signals.

In contrast to its rapid depolarization of POMC neurons, leptin hyperpolarizes and decreases the firing rate of NPY/AgRP neurons (12,13; and E.E. Jobst and M.A. Cowley, unpublished). Although definitive evaluation of the signal transduction pathway of leptin in NPY/AgRP...
Box 1. Peripheral signals regulating long-term body weight homeostasis

**Leptin**
Substantial evidence has shown that leptin, a circulating anorexigenic hormone produced by white adipocytes [61], has a fundamental role in a neuroendocrine feedback loop involved in maintaining energy homeostasis [62]. In obese leptin-deficient mice, exogenous administration of leptin effectively reduces hyperphagia and obesity [63]. Conversely, obese mice that are deficient in the signaling form of the leptin receptor do not respond to leptin [63,64].

Leptin receptors (ObRb) are highly expressed in regions of the hypothalamus that mediate energy homeostasis [65,66]. To access these receptors, peripheral leptin is transported across the blood–brain barrier to reach areas distal to circumventricular organs [25]. In the arcuate nucleus of hypothalamus (ARH), leptin exerts some of its effects by acting through ObRb receptors on at least two distinct populations of neurons. Leptin reduces the mRNA expression of two potent orexigenes, neuropeptide Y (NPY) [66] and agouti-related protein (AgRP) [67]. By contrast, leptin increases the expression of proopiomelanocortin (POMC) mRNA, which might promote the release of the peptide α-melanocyte-stimulating hormone (α-MSH), a potent anorexigen at central melanocortin-4 (MC-4) receptors [68].

**Insulin**
Insulin, a pancreatic hormone that is essential for stimulating glucose uptake and metabolism in peripheral tissues, was the first identified circulating signal proposed to be involved in energy homeostasis [69]. Central administration of insulin decreases food intake and body weight, and insulin receptors are concentrated in brain regions that are involved in controlling the intake of food (reviewed in Ref. [15]).

Electrophysiology of ghrelin

**Ghrelin in the ARH**
Although evidence indicates that circulating ghrelin might activate vagal afferents and subsequent brainstem pathways [17,18], its role in feeding regulation seems to be predominantly mediated by its actions in the ARH (Box 2) [19]. In hypothalamic slices containing the ARH, ghrelin dose-dependently stimulates electrical activity in most ARH neurons that are inhibited by leptin [12,13].

Recently, we and others have shown that ghrelin directly activates NPY/AgRP neurons [12,13]. Bath application of ghrelin was found to increase the spontaneous action potential frequency of NPY/AgRP neurons by 300%. Conversely, the same dose of ghrelin caused a 50% decrease in the spontaneous activity of POMC neurons. The latter effect on POMC neurons was abolished by the blockade of Y1 (the NPY receptor subtype on POMC neurons) and GABA_A receptors, demonstrating that the inhibitory effect of ghrelin on POMC neurons is dependent on Y1-receptor activation.
Box 2. Peripheral signals regulating short-term body weight homeostasis

**Ghrelin**

Ghrelin was the first peripheral orexigenic signal to be identified [70]. Ghrelin is the endogenous ligand of the growth hormone secretagogue receptor (GHS-R), which is densely expressed in pituitary and hypothalamic nuclei [71]. Although ghrelin is expressed in almost all tissues, its expression is highest in the stomach, where its secretion from A-like cells is upregulated during fasting and hypoglycemia [72]. Early studies have also detected ghrelin expression in the brain [70]. So far, however, the specific CNS expression pattern of ghrelin has been characterized only in the telencephalon and hypothalamus [12].

Whereas leptin inhibits food intake and increases energy expenditure, ghrelin produces a positive energy balance by promoting food intake and decreasing energy expenditure [21,73]. Expression and secretion of ghrelin decrease with feeding and obesity [74,75] and increase with caloric restriction and weight loss [76,77]. Accumulating evidence also suggests that a decrease in circulating ghrelin is an important factor in the long-term success of weight control after some bariatric surgical procedures [78].

**PYY**

Neuropeptide PYY comprises 36 amino acids and is released from enteroeendocrine L cells that line the distal small bowel and colon [79]. About 40% of PYY is enzymatically cleaved in the gastrointestinal tract, resulting in a shortened form of PYY containing 34 amino acids termed PYY3–36 [80]. PYY is released into the circulation in response to food intake [81], and PYY3–36 is the predominant form of PYY in postprandial human plasma [80]. Its release is proportional to calorie intake, but is also influenced by meal composition, whereby fat intake stimulates higher plasma concentrations of PYY [81]. PYY shares 70% structural homology with neuropeptide Y (NPY). Whereas PYY1–36 binds with high affinity to all NPY receptors, PYY3–36 shows moderate selectivity for the NPY Y2 receptor. This receptor is an inhibitory presynaptic receptor that is highly expressed on NPY neurons, but barely present on neighboring proopiomelanocortin (POMC) neurons in the arcuate nucleus of hypothalamus (ARH) [26]. Indeed, only 3% of POMC neurons in the ARH express Y2 receptors [26,82].

Intraperitoneal administration of PYY3–36 reduces food intake and body weight gain both in normal rodents [27] and in diverse rodent models of metabolic disease [83]. The anorectic effects of PYY3–36 are also evident in humans. Two hours after receiving an infusion of PYY3–36, volunteers with normal weight [27] and with obesity [84] were found to have reduced their food intake by more than 30% without a change in gastric emptying [27]. PYY acts centrally to inhibit gastric emptying and motility by a vagally mediated mechanism – the so-called ‘ileal brake phenomenon’ [33,85]. Although PYY is also expressed in the brain, its functions are unknown [86]. Because intracerebroventricular injection of PYY to sites other than the ARH strongly stimulates hyperphagia, the anorectic effects of PYY might depend on specific access to ARH neurons (and perhaps the area postrema).

on the presynaptic activation of NPY/AgRP neurons. In this manner, ghrelin functions like the neuropeptide PYY3–36 (discussed below), but results in opposite effects. Thus, ghrelin directly activates orexigenic NPY/AgRP neurons and indirectly inhibits anorexigenic POMC neurons.

Recently, focus has been directed at elucidating the signal transduction mechanisms of ghrelin in ARH neurons. Using ratiometric fura-2 fluorescence imaging of isolated single ARH neurons, Kohno et al. [20] have shown that ghrelin dose-dependently increases cytosolic Ca2+ in a third of ARH neurons. Roughly 80% of these ghrelin-responsive neurons were found to be immunoreactive for NPY. Inhibitors of protein kinase A and N-type Ca2+ channels significantly attenuated the ghrelin-induced increases in cytosolic Ca2+, suggesting that ghrelin directly interacts with NPY/AgRP neurons to induce Ca2+ signaling via protein kinase A and N-type Ca2+-channel-dependent mechanisms [20]. These observations are partially consistent with recent electrophysiological evidence showing that ghrelin, as well as orexin (discussed below), produces a bursting pattern in NPY/AgRP neurons that is driven by low-threshold (T-type) Ca2+ conductances and transient outwardly rectifying K+ conductances [13].

Both studies indicate that ghrelin induces increases in cytosolic Ca2+ in NPY/AgRP neurons in the ARH; however, the electrophysiology data demonstrate that ghrelin activates a low-voltage-activated T-type Ca2+ conductance, whereas the Ca2+ imaging data support the activation of N-type Ca2+ channels by ghrelin. The reasons for this discrepancy are unclear. In the electrophysiology experiments, T-type Ca2+ channels were found to be responsible for underlying membrane potential oscillations in a subtype of ARH neurons called ARH pacemaker neurons. These membrane oscillations were required for ghrelin-induced bursts of action potentials. The Ca2+ increases recorded in fura-2 imaging often result from neuronal depolarization and are therefore a good indicator of neuronal activation; however, it is not known what activity pattern ghrelin causes in these neurons. Thus, it is impossible to determine from the Ca2+ imaging data whether the activation of N-type Ca2+ channels by ghrelin occurred in the same subset of NPY/AgRP pacemaker neurons identified in the electrophysiology experiments [13].

**Ghrelin in the brainstem**

Although the ARH has been the principal focus of research on the central actions of ghrelin, the receptor for ghrelin – the growth hormone secretagogue receptor (GHS-R) – is expressed in the caudal brainstem [21], and administration of ghrelin directly into the dorsal vagal complex (DVC) produces hyperphagia with sensitivity comparable to that reported for the ARH [21]. The DVC, a hindbrain autonomic center that includes the nucleus tractus solitarius (NTS), dorsal motor nucleus of the vagus (DMV) and area postrema [22], functions as the central hub that provides parasympathetic control over the gastrointestinal tract and coordinates digestive functions. Perhaps surprisingly, then, peripheral injections of ghrelin (at doses that increase food intake) selectively induce c-Fos expression only in ARH neurons and not in other hypothalamic, limbic, pontine or medullary brain structures [23,24].

Although it is possible that peripheral ghrelin has inhibitory actions in the caudal brainstem (and thus would not be detected by c-Fos expression), there are no electrophysiological studies reporting the effect of ghrelin on hindbrain neurons. Nevertheless, recent evidence might curtail debate over whether the effect of ghrelin on food intake has a direct action on the hindbrain. Data from Chen et al. [19] firmly support the assertion that peripheral ghrelin stimulates feeding by acting through
ARH neurons. The primary discovery of this group is that mice lacking the genes encoding NPY and AgRP have no response to peripheral ghrelin-induced feeding. Crucially, Chen et al. have shown that ghrelin functions through NPY/AgRP neurons (in the ARH), and that ghrelin only works if NPY and AgRP are expressed, thereby identifying NPY/AgRP neurons as requisite mediators of ghrelin-stimulated feeding.

At face value these results seem difficult to reconcile with studies showing that administration of ghrelin directly into the brainstem produces hyperphagia [21], and that the blockade of afferent vagal neurotransmission or vagotomy prevents the response to peripheral ghrelin but has no effect on the feeding response to central administration of ghrelin [17,18]. One way to resolve the issue over whether brainstem circuitry is required for ghrelin-induced feeding would be to examine the extent of c-Fos expression that is induced in NPY/AgRP neurons by peripheral ghrelin in decerebrate animals. We anticipate that, if a ghrelin hindbrain relay is necessary for ghrelin-induced activation of NPY/AgRP neurons in the ARH, then decerebration, in which all brainstem to hypothalamic connectivity is severed, will prevent the c-Fos-mediated activation of NPY/AgRP neurons. At present, we can confidently state that the primary site of the central action of ghrelin is the ARH but we cannot rule out the possibility that it has a complementary action in the brainstem.

Electrophysiology of PYY₃–₃₆

PYY in the ARH

Few studies have examined the central electrophysiological actions of the neuropeptide PYY (Box 2). The direct effects of leptin and ghrelin on ARH neurons suggest that these neurons might have access to circulating hormones, either via specific transport mechanisms or via diffusion from the median eminence – a circumventricular organ that has a more permeable blood–brain barrier [23].

On the basis of the differential distribution of the NPY Y2 receptor on ARH neurons [26], we and our colleagues [27] proposed that activation of the Y2 receptor by PYY₃–₃₆ would inhibit NPY/AgRP neurons and thereby disinhibit POMC neurons. In recordings from the ARH in hypothalamic slices, we found that PYY₃–₃₆ depolarized POMC neurons and increased the frequency of spontaneous
Box 3. Central mediators of energy homeostasis

**Orexin**
The orexins (also known as the hypocretins) comprise a pair of peptides, orexin A and orexin B, that are derived from the same precursor peptide (prepro-orexin) by proteolytic processing. The orexins are expressed by a population of neurons in the lateral hypothalamic area (LHA) and perifornical area [34], and to a lesser extent in the dorsomedial nucleus of the hypothalamus (DMH) [39].

The orexins bind and activate two closely related G-protein-coupled receptors, termed OX1R and OX2R. OX1R is relatively selective for orexin A, whereas OX2R is a nonselective receptor for both orexin A and orexin B [35]. Considerable evidence suggests that OX1R is coupled to activation of the Gq subtype of GTP-binding proteins, leading to stimulation of phospholipase C and protein kinase C [87,88]. OX2R might be coupled to Gs proteins in cultured pituitary somatotropes [89], and recent evidence suggests that this receptor is coupled to pertussis-toxin-sensitive Gi- and/or Go-binding proteins in proopiomelanocortin (POMC) neurons in the arcuate nucleus of hypothalamus (ARH) [43].

In contrast to the fairly restricted distribution of orexin-immunoreactive somas, the expression of orexin receptors is extensively distributed throughout the cerebral cortex, limbic system, posterior hypothalamus, thalamus and brainstem (Figure 3) [34,46,47,90]. OX1R and OX2R show, however, a markedly different distribution [91,92]. In the hypothalamus, expression of OX1R mRNA is largely restricted to the ventromedial nucleus of hypothalamus (VMH) and DMH, whereas expression of OX2R mRNA is high in the paraventricular nucleus of hypothalamus (PVH), VMH and ARH, as well as in the mammillary nuclei [92]. When administered centrally, orexin increases feeding, locomotion and wakefulness [35,93,94], consistent with activation of c-Fos in areas mediating these actions [46]. By contrast, fasting increases the levels of prepro-orexin mRNA [35].

**Melanin-concentrating hormone**
Melanin-concentrating hormone (MCH) is synthesized primarily in neurons in the LHA [49], but in a population that is distinct from the one that produces orexin [95]. MCH binds to a G-protein-coupled receptor termed either somatostatin-like (SCL-1) or MCH-1R [52]. Humans also express a second receptor for MCH, MCH-2R, that is not present in the rodent brain [96]. Expression of MCH-1R is widespread in the brain. In the hypothalamus, MCH-1R is found in VMH, ARH and DMH hypothalamic nuclei [52,53,58], as well as in the LHA [58]. In humans, MCH-2R is densely expressed in the ARH and the VMH [97]. Similar to orexin, MCH is an important regulator of feeding behavior [98]. Central administration of MCH stimulates food intake, whereas fasting increases MCH expression [98]. MCH-deficient mice have lower body weights owing to reduced feeding and enhanced metabolism, despite their reduction in both leptin and POMC mRNA in the ARH [99]. Conversely, transgenic overexpression of MCH in the LHA increases the body weight of mice fed on standard and high-fat diets [100].

Notably, both orexin and MCH in the LHA might be involved in communicating the hedonic or rewarding aspects of feeding [6,101]. Feeding is powerfully influenced by pleasure and reward [6], and the LHA is involved in feeding, arousal and motivated behaviors [101]. LHA neurons are well placed for a role in the reward pathway. Orexin receptors are expressed at high levels in limbic regions such as the ventral tegmental area and the locus coeruleus [92,102]. Likewise, it is possible that MCH signaling from the LHA to the nucleus accumbens, a principal reward region in the brain, is involved in hedonic aspects of feeding.

**Electrophysiology of orexin**

**Orexin in the lateral hypothalamic area**
Owing to the widespread distribution of orexin-immunoreactive axons (Figure 3), the actions of orexin have been investigated in several feeding-related regions of the CNS (Box 3). So far, orexin action has been found to be excitatory in all of the regions analyzed with the notable exception of the ARH.

The earliest studies on cultured neurons showed that orexin was excitatory to hypothalamic neurons, but not to hippocampal neurons [34]. In hypothalamic slices, the effects of orexin were first assessed in the lateral hypothalamic area (LHA) itself. The LHA contains orexin neurons and glucose-sensitive neurons (GSNs), both of which are stimulated by hypoglycemia and are implicated in hypoglycemia-induced feeding [35,36]. When applied to hypothalamic brain slices, orexin A was found to increase spontaneous action potentials by 500% and to cause a rapid, long-lasting tetrodotoxin-resistant depolarization of glucose-sensitive neurons, indicating that it has a direct postsynaptic effect on GSNs in the LHA [37]. Confocal microscopic evaluation has shown that orexin-immunoreactive axons are often intertwined with GSN dendrites, establishing putatively synaptic contacts [37].

**PYY in the brainstem**
Binding sites with affinity for PYY, specifically Y1 and Y2 receptors, have been identified in the DVC [28,29], which suggests that this region might have access to PYY in vivo. A portion of the DVC (neurons in the area postrema) lies outside the blood–brain barrier, and these neurons might respond to circulating gastrointestinal hormones and relay these signals to the NTS and parabrachial nucleus [30].

Using whole-cell patch clamp recordings from identified GI-projecting neurons of the rat DMV, Browning and Travagl [31] have shown that the main effect of PYY3–36 in the DVC is an inhibition of glutamatergic synaptic transmission between the NTS and the DMV. This PYY-induced decrease in excitatory transmission between the NTS and the DMV results from activation of presynaptic Y2 receptors. These results are consistent with earlier studies showing that PYY-mediated inhibitory actions on gastrointestinal motility result from interactions with brainstem Y2 receptors [32,33].

Thus, the effects of circulating PYY3–36 after ingestion of a meal seem to be mediated by the Y2 receptor in both the brainstem and the ARH. Notably, the same peptide suppresses digestive functions by decreasing ‘excitatory’ tone in the DVC and suppresses appetite by decreasing ‘inhibitory’ tone in the ARH.

**Review**

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To assess the response of orexin neurons to feeding-related signals, Yamanaka et al. [38] generated transgenic mice expressing a fusion protein of orexin and enhanced green fluorescent protein (orexin–EGFP). In dispersed cultures and in hypothalamic slices, orexin–EGFP neurons depolarized in response to glutamate and ghrelin (a peripheral appetite-stimulating peptide) and hyperpolarized in response to GABA and leptin (a peripheral appetite-inhibiting hormone). Similarly, orexin–EGFP neurons had a predictable response to changes in extracellular glucose concentration; namely, they depolarized in response to falling levels and hyperpolarized in response to rising levels. Notably, insulin had no effect on orexin–EGFP neurons [38].

Thus, both the direct responsiveness of orexin neurons to feeding-related signals and the subsequent activation of GSNs by orexin might mediate the onset of hypoglycemia-induced feeding and contribute to the appetite-stimulating effect of orexin A.

Orexin in the ARH and VMH

Given that orexin-immunoreactive axon terminals are exceptionally abundant in the ARH [39] and that the orexin receptor OX1R is located in both POMC- and NPY-expressing neurons in the ARH [40], several studies have evaluated the electrophysiological effects of orexin in the ARH [13,41,42]. Early extracellular recordings showed that orexin increases the firing rate of most ARH neurons [42]. Recently, van den Top et al. [13] assessed the effect of orexin on the activity pattern of NPY/AgRP neurons in the ARH. Importantly, these investigators noted that the application of orexin, as well as ghrelin (see above), produced bursts of action potentials corresponding to underlying membrane potential oscillations. This bursting pattern, driven by low-threshold Ca²⁺ conductances and transient outwardly rectifying potassium conductances [13], has been shown to be a more effective stimulus for neuropeptide release than has repetitive firing [9]. Crucially, the effects of orexin on GABAergic NPY/AgRP neurons would preferentially release the two potent orexigen NPY and AgRP.

Using similar techniques, Burdakov et al. [41] have also reported that a subset of GABAergic ARH neurons is directly activated by orexins. They found that the concentration dependency of this activation was similar for orexin A and B, suggesting involvement of the orexin receptor OX2R. Neuronal activation by orexins was dependent on the mobilization of Ca²⁺ from intracellular stores, which Burdakov et al. concluded was dependent on an Na⁺/Ca²⁺ exchanger. Although this conclusion is one potential interpretation, it was based on data showing that an Na⁺/Ca²⁺ inhibitor reversed the actions of orexin.
Because current–voltage relationships before and after the addition of the pump inhibitor were not shown, however, at present we are not convinced that this is the mechanism by which orexin activates NPY neurons in the ARH.

What about the effects of orexin on POMC neurons in the ARH? Similar to their synaptic contacts onto NPY/AgRP neurons in the ARH [39,43], orexin fibers also make synaptic contacts onto POMC neurons [43,44]. These orexin–POMC synapses are primarily symmetrical at the ultrastructural level, which suggests that orexin inhibits POMC neurons [44]. Although no electrophysiological evidence supports this presumption or assertion, it is consistent with the rationale that an orexinergic peptide would inhibit the activity of POMC neurons [44]. Although no electrophysiological testing, by concurrently inhibiting a specific K+ conductance consistent with mediation by OX1R, orexin decreases cytosolic Ca2+ in POMC neurons. Orexin increases cytosolic Ca2+ biphasically, with pharmacological properties consistent with mediation by OX1R, phospholipase C, inositol (1,4,5)-trisphosphate and protein kinase C signaling pathways. By contrast, the orexin-mediated decrease in cytosolic Ca2+ in POMC neurons is most probably mediated by OX2R (given the comparable responses to orexin A and orexin B) and involves the activation of Gi2 and/or Gi3, GTP-binding proteins (given the sensitivity to pertussis toxin). Future electrophysiological recordings of the effects of orexin on POMC neurons in hypothalamic slice preparations will be helpful to corroborate these Ca2+ imaging data.

Shiraishi et al. [45] have compared the effects of orexin and leptin on extracellular neuronal activity in the VMH. They found that orexin significantly decreases the activity of gluoresponsive neurons (GRNs) – neurons that are normally excited by rising glucose concentrations. Conversely, leptin increases the activity of GRNs in comparison to non-GRNs. These data have been corroborated by Ca2+ imaging data showing the reciprocal effects of these peptides on cytosolic Ca2+; namely, orexin decreases cytosolic Ca2+ and leptin increases cytosolic Ca2+ in GRNs in the VMH [43]. Thus, orexins might stimulate feeding by acting in the ARH to activate NPY/AgRP neurons and to inhibit POMC neurons, while simultaneously inhibiting GRNs in the VMH. At this stage, future experiments in the ARH and VMH must be done to complete the emerging picture of how leptin and orexin act reciprocally to mediate feeding status.

Orexin in the brainstem

The NTS is a brainstem region that has a central role in many autonomic functions and receives dense orexin innervation from the LHA [46,47]. Indeed, orexin A has been found to depolarize more than 90% of NTS neurons tested, by concurrently inhibiting a specific K+ conductance and activating a nonselective cationic conductance [48]. The NTS sends efferent output to many hypothalamic nuclei as well as to the DMV, whose efferent parasympathetic visceromotor fibers innervate the whole gastrointestinal tract (Figure 4). Whether the actions of orexin in the NTS have a direct role in feeding regulation is, however, currently unknown.

Electrophysiology of melanin-concentrating hormone

Melanin-concentrating hormone in the LHA

The highest density of melanin-concentrating hormone (MCH)-immunoreactive axons is found in the LHA, and many of these axons make synaptic contacts with other LHA neurons [49]. In cultured synaptically coupled LHA neurons, MCH potently inhibits synaptic activity (Box 3) [50]. Although MCH has no direct effect on resting membrane potential of LHA neurons, its application reduces and sometimes abolishes spontaneous action potentials. Notably, Gao and van den Pol [50] have suggested that the mechanism underlying the inhibition of spontaneous firing by MCH might be related to an indirect effect on neurotransmission because synaptic transmission mediated by glutamate (and GABA to a lesser extent) is inhibited by MCH.

A powerful mechanism for controlling neurotransmitter release is the modulation of Ca2+ influx via voltage-dependent Ca2+ channels at presynaptic terminals [51]. In a subsequent study, Chen and van den Pol [51] showed that the inhibitory action of MCH in LHA neurons is due to the inhibition of voltage-dependent Ca2+ currents (with L- and N-type channels accounting for most of the voltage-activated current) via G-protein pathways that are sensitive to pertussis toxin [51].

MCH in the ARH and VMH

Given the density of both MCH-immunoreactive fibers [49] and the MCH receptor MCH-1R [52,53] in the VMH and ARH, Davidowa et al. [54] studied the effect of MCH on single-unit activity in hypothalamic slices containing the VMH and ARH in normal and overweight rats. In slices from normally fed or food-deprived rats of normal weight, 20–40% of tested neurons responded to the application of MCH, showing either an increase or decrease in spontaneous activity. By contrast, MCH produced consistent responses in neurons from overweight rats: it predominantly activated ARH neurons and inhibited VMH neurons.

Davidowa et al. [54] proposed that MCH supports a shift in energy balance to a higher level of body weight in overweight animals by simultaneously activating NPY neurons in the ARH (to promote feeding) and inhibiting neurons in the VMH (to reduce energy expenditure) [54]. The signaling mechanisms that promote or facilitate this switch in energy balance are completely unknown. It is also worthwhile noting that the MCH-activated neurons were identified as NPY neurons solely by their location in the ARH and not by immunocytochemical characterization.

MCH in the paraventricular nucleus of hypothalamus

The paraventricular nucleus of hypothalamus (PVH), in particular its parvicellular region, is involved in regulating body weight [55,56]. This region has long been
identified as a ‘satiety center’, because lesions within it produce hyperphagic obesity [57]. The MCH receptor MCH-1R is abundantly expressed in the PVH [58], and Davidowa et al. [59] have studied the effect of MCH on single-unit activity in hypothalamic slices containing the PVH, mainly its medial parvicellular part, from normal and overweight rats. In slices from rats of normal weight, the application of MCH did not have a predictable response and excited or inhibited equal numbers of neurons. By contrast, neurons from overweight rats were predominantly inhibited by MCH [59]. Because the output from the PVH is predominantly catabolic (Figure 5) [60], we anticipate that an inhibition of PVH neurons by MCH would continue to support the already existing positive energy balance in overweight rats. The signaling pathways in normal and overweight animals need to be examined further to understand how states in energy balance can change the effects of a single neuropeptide.

Future directions
Our understanding of feeding circuits from electrophysiology experiments has led to a working circuit diagram including several nuclei in the hypothalamus and brainstem. Our goal is to use this circuit as a guide to test putative feeding-related signals and potential therapeutic drugs at relevant target sites. Future experiments need to test the convergence of multiple feeding-related signals, because most studies have assessed the effects of single agents on neuronal activity and on energy homeostasis. The development of models that facilitate recording from specific cell types, coupled with tracing studies, will accelerate the mapping of these circuits. It is also important to test the function of these circuits in altered physiological states – for example, in obese animals and animals that are becoming obese. As we learn more, we will no doubt expand and challenge our current understanding of feeding circuits.

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